Furan formation during storage and reheating of sterilized vegetable purées

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To this day, research for furan mitigation has mostly targeted the levels of food production and handling of prepared foods by the consumer. However, part of the furan concentrations found in commercially available food products might originate from chemical deterioration reactions during storage. A range of individual vegetable purées was stored at two different temperatures to investigate the effects of storage on the furan concentrations of shelf-stable, vegetable-based foods. After 5 months of storage at 35 °C (temperature-abuse conditions), a general increase in furan concentrations was observed. The furan formation during storage could be reduced by storing the vegetable purées at a refrigerated temperature of 4 °C, at which the furan concentrations remained approximately constant for at least 5 months. Following storage, the vegetable purées were briefly reheated to 90 °C, to simulate the effect of the final preparation step before consumption. As contrary to storage, furan concentrations decreased as a result of evaporative losses. Both refrigerated storage and the reheating step prior to consumption showed the potential of mitigation measures for furan formation in vegetable-based foods (e.g. canned vegetables, ready-to-eat soups, sauces or baby foods). Next to furan, the vegetable purées were analyzed for 2- and 3-methylfuran. Tomato was very susceptible to the formation of both alkylated derivatives of furan, as opposed to the other vegetables in this study. Methylfuran concentrations rapidly decreased during storage, which was contrary to the results observed for furan.

Keywords: furan; methylfuran; vegetable; sterilization; storage; reheating.

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Introduction

Furan (C_4H_4O) is a small organic molecule with high volatility. In 1995, furan was classified as 'possibly carcinogenic' to humans after it was proven to be carcinogenic in rats and mice (International Agency for Research on Cancer (IARC) 1995). Furan can be formed in a variety of heat-treated foods. The highest concentrations are found in coffee products and canned or jarred foods, products that are subjected to an intensive heat treatment for roasting or sterilization purposes (US Food and Drug Administration (FDA) 2009; European Food Safety Authority (EFSA) 2011). In the literature, many ways leading to the formation of furan have been reported, the major precursors being sugars (alone or in combination with amino acids), ascorbic acid and unsaturated fatty acids, followed by amino acids and carotenoids (Locas & Yaylayan 2004; Becalski & Seaman 2005; Fan 2005; Mark et al. 2006; Limacher et al. 2007; Limacher et al. 2008; Owczarek-Fendor et al. 2010; Owczarek-Fendor et al. 2011; Van Lancker et al. 2011; Huang et al. 2011; Owczarek-Fendor et al. 2012). Vegetable-based foods (e.g. canned vegetables, ready-to-eat soups, sauces or baby foods) are particularly susceptible to furan formation, because they are natural mixtures of all known furan precursors, combined with a low acidity that implies a sterilization treatment to obtain shelf-stable products.

A recent risk evaluation by the Joint FAO/WHO Expert Committee on Food Additives (2011) has indicated a human health concern for furan and consequently, actions should be taken to minimize exposure to an acceptable level. The most obvious target for furan mitigation is the thermal processing step for preservation, e.g. by optimization of the conventional heating process or application of an alternative processing technique. In this context, high-pressure high-temperature (HPHT) processing has recently presented itself as an interesting alternative for furan reduction in vegetable-based foods (Palmers et al. 2014). Following a HPHT treatment (600 MPa, 117 °C, F_0 = 5 min), the furan concentrations of a wide range of vegetable purées decreased to levels close to the analytical limits (1-2 ng/g purée). At this moment, the specific applications of HPHT processing are still under research, and equipment and operating costs might limit its use to high value-added foods. For many other products, conventional thermal processing would remain the standard technique of sterilization. For these

78 products, mitigation strategies aiming at an intervention in the reaction mechanism of furan formation
79 (e.g. by changing the product composition) might have the best potential for furan reduction. As a first
80 step towards product optimization of vegetable-based foods, vitamin C and sugars were identified as the
81 major precursors and possible targets for furan mitigation (Palmers et al. 2014). In any way, the
82 reduction of furan concentrations in thermally treated foods has proven to be challenging, both because
83 of the microbial safety standards to comply with, and the variety of possible furan precursors. Therefore,
84 researchers have been looking for other, complementary ways to reduce furan concentrations. Some
85 researchers have targeted the consumer level, however also with varying success. Up to 50% of the
86 initial furan concentrations could be lost by reheating and/or stirring of the products (Zoller et al. 2007;
87 Roberts et al. 2008; Kim et al. 2009). Nevertheless, all consumer advice is considered premature at this
88 point, because the reduction in furan concentrations was not consistently observed in other studies
89 (Hasnip et al. 2006; Lachenmeier et al. 2009).
90 Following sterilization, most vegetable-based food products are considered to be microbiologically
91 stable, and hence can be stored for a long time (~years) at temperatures exceeding room temperature.
92 However, regardless of the product or the applied intensity of the preservation process, there is always a
93 certain potential left for chemical changes during subsequent storage. This chemical instability of heat-
94 treated foods seems to be ignored in the literature concerning furan as a process-induced contaminant.
95 Theoretically, storage can result in both an increase (due to enhanced degradation of its precursors and
96 reaction products) or a decrease of the furan concentration (due to degradation reactions of furan itself),
97 which is important information in the context of furan mitigation. The product stability depends on
98 numerous intrinsic (e.g. water activity, pH) and extrinsic parameters (e.g. temperature, time, light)
99 (Narayan 1997), making it difficult to predict whether the changes in product composition during
100 storage will have an observable effect on the level of furan concentration. Regarding vegetable-based
101 foods, major furan precursors such as vitamin C and sugars are known to be degraded during storage
102 (Taub & Singh 1997). As a result, these products might be identified as possible risk matrices with
103 regard to furan formation during storage. Therefore, the objective of the present study was to gain

104 insight into the effects of storage and reheating on the furan concentrations of sterilized, vegetable-
105 based foods. As recently proposed by Becalski et al. (2010) and adopted in the recommendations of the
106 Joint FAO/WHO Expert Committee on Food Additives (2011), also the concentrations of 2- and 3-
107 methylfuran were monitored. Both alkylated derivatives of furan might be of toxicological interest,
108 since animal studies have shown that they can be metabolically activated in a similar way as furan (Gill
109 et al. 2014a; Gill et al. 2014b).
110
111 **Material and methods**
112 ***Preparation of the vegetable purées***
113 Seven vegetables were freshly bought at a local supplier. The selection included broccoli (*Brassica*
114 *oleracea* Italica Group), orange carrot (*Daucus carota* 'Nerac'), pea (*Pisum sativum*, variety unknown),
115 potato (*Solanum tuberosum* 'Artemis'), pumpkin (*Cucurbita maxima* 'Hokkaido'), tomato (*Solanum*
116 *lycopersicum* 'CLX 38197') and red beet (*Beta vulgaris* 'Pablo'). All vegetables are commonly used
117 ingredients of ready-to-eat foods, such as jarred baby foods, soups and sauces, products found to be
118 susceptible to furan formation in recent monitoring studies (US Food and Drug Administration (FDA)
119 2009; European Food Safety Authority (EFSA) 2011). Furthermore, the selected vegetables differ in
120 their composition regarding furan precursors. The vegetables were carefully washed and cut into
121 standardized pieces of approximately 1 cm thickness, before vacuum-packing in low-density
122 polyethylene bags. To assure that all the changes observed during storage or reheating were chemical,
123 the vegetable pieces were blanched at 95 °C for 8 min in a water bath (WBU 45, Memmert, Schwabach,
124 Germany). The blanching conditions were validated using a qualitative and quantitative peroxidase test
125 (Adebooye et al. 2008; Vervoort et al. 2012). After blanching, the plastic bags were immediately cooled
126 in iced water for 10 min, frozen in liquid nitrogen, and stored in a freezer at -40 °C. Prior to purée
127 preparation, the vegetable pieces were thawed overnight in a cold room at 4 °C. The vegetable pieces
128 were mixed with a standardized amount of deionized water and blended for 1 min to obtain seven
129 individual vegetable purées. Finally, the vegetable purées were subjected to a high pressure

130 homogenization (GEA Niro Soavi, Parma, Italy) at 100 MPa, to ensure the stability of the purées during
131 the thermal treatment for sterilization and subsequent storage of the samples.
132
133 ***Thermal sterilization***
134 The individual vegetable purées were subjected to a thermal sterilization in a static Steriflow pilot
135 retort (Barriquand, Roanne, France). Due to their inert nature, glass jars (100 ml volume, 95 mm height
136 and 45 mm diameter) were used as sample holders. The jars were filled with 85 ± 0.5 g of vegetable
137 purée and then closed with metal lids. Next, they were loaded into the retort and sterilized at a retort
138 temperature of 117 °C. The holding time (±30 min) was calculated in advance to obtain an industrially
139 relevant process value $F_{121.1\,^{\circ}C}^{10\,^{\circ}C}$ (F_0) = 5 min in the coldest point of the product. Temperature profiles in
140 the retort and in the product were recorded using type T thermocouples (Ellab, Hilleroed, Denmark)
141 (results not shown). After sterilization, the glass jars were immediately transferred to iced water to
142 reduce further chemical reactions. A sample was taken for (methyl)furan analysis, and the remaining
143 jars were used for storage and reheating experiments.
144
145 ***Storage conditions***
146 After sterilization, the vegetable purées were placed in incubators for storage at constant temperature,
147 and protected from light. To select appropriate storage conditions, the principles of accelerated shelf life
148 testing (Toledo 2007) were applied. From a worst-case perspective, part of the sterilized vegetable
149 purées were stored at 35 °C for 5 months. Based on a theoretical temperature coefficient of (Q_{10}) of 2,
150 these storage conditions should correspond to a storage time of approximately one year at 20 °C. To
151 evaluate the potential of storage at optimal (refrigerated) storage conditions, the other part of the
152 vegetable purées were stored at 4 °C for 5 months. After storage, the glass jars were again transferred to
153 iced water to reduce further chemical reactions. A sample was taken for (methyl)furan analysis, and the
154 remaining jars were used for reheating.
155

156 ***Reheating procedure***

157 At every important step of the storage experiment (after sterilization and after 5 months of storage at 4
158 and 35 °C), a sample of each vegetable purée was taken for reheating. This sample consisted of 8 g
159 vegetable purée which was transferred into a small glass tube (10 ml, approximately 1 cm headspace), to
160 allow fast heating up and cooling rates, simulating the heating rates applied in microwave heating. From
161 a worst-case perspective, the glass tubes were closed with a plastic cap. This way, a possible loss of
162 furan during the reheating procedure was reduced as much as possible. The vegetable purées were
163 briefly reheated by placing them in a water bath (WBU 45, Memmert, Schwabach, Germany) at 95 °C.
164 Temperature profiles in the water bath and at the coldest point of the product were recorded using type
165 T thermocouples (Ellab, Hilleroed, Denmark) (results not shown). When a product temperature of 90 °C
166 was reached, the samples were removed from the water bath and immediately transferred to iced water
167 to reduce further chemical reactions. Depending on the type of vegetable purée, this resulted in a heating
168 time between 3 and 4 min. All the reheated vegetable purées were analyzed for (methyl)furan.
169
170 ***Quantitation of furan and methylfuran***

171 Furan was analyzed with solid phase microextraction coupled to gas chromatography-mass
172 spectrometry (SPME-GC-MS), using furan-d_4 as an internal standard. The analytical procedure is
173 described in detail elsewhere (Palmers et al. 2014), and will only be discussed here in brief. Standard
174 stock (ca. 2.5 mg/ml in methanol) and working solutions (ca. 0.25 µg/ml in deionized water) of internal
175 standard were obtained by making a serial dilution of furan-d_4 (98%, Sigma Aldrich, Saint Louis,
176 Missouri) in closed headspace vials of 10 ml. Both the amount of solvent and the amount of solution
177 added were determined by differential weighing and the exact concentration of furan-d_4 was calculated
178 from the respective masses. The samples were prepared for analysis by weighing 2.5 g of the cooled
179 purée in an empty 10 ml headspace vial with a PTFE/silicone septum seal. Before the vial was
180 completely closed, 2.5 ml of a saturated NaCl solution was added to the purée and the mixture was
181 further diluted with deionized water to obtain a standardized total volume of 6 ml. After sealing, 100 µl

182 of the internal standard working solution was added with a chilled gastight syringe. The exact amounts
183 were determined again by differential weighing. All operations were done in a closed and refrigerated
184 sample preparation box (MPR-311D(H), Sanyo, Moriguchi, Japan) and the samples were stored at a
185 temperature of 10 °C until analysis.
186 The analyses were carried out using an Agilent 7890A GC and an Agilent 5975C MS (Keysight
187 Technologies, Santa Rosa, California), equipped with a CTC Combi PAL autosampler (CTC Analytics,
188 Zwingen, Switzerland). The SPME fiber (Supelco, Bellefonte, Pennsylvania) had a 85 μm
189 carboxen/polydimethylsiloxane (CAR/PDMS) sorptive coating, which was exposed to the headspace of
190 the samples for 15 min at 30 °C. After headspace extraction, the fiber was transferred to the GC
191 injection port, where the adsorbed compounds were thermally desorbed for 1 min at 200 °C. After each
192 run, the fiber was thermally cleaned for 2 min at 300 °C in the conditioning station of the autosampler.
193 The volatiles were injected in the splitless mode and subsequently separated on a HP-PLOT Q column
194 (30 m × 320 μm, 20 μm film thickness, Keysight Technologies, Santa Rosa, California), using helium
195 as the carrier gas at a constant flow rate of 2 ml/min. The column oven was programmed at a starting
196 temperature of 40 °C, which was retained for 4 min, after which it was elevated to 160 °C at a rate of 40
197 °C/min, followed by a second ramp to 220 °C at 5 °C/min. After 1 min at the final temperature, the oven
198 was cooled again to the initial temperature. Mass spectra were obtained by electron ionisation (EI) at 70
199 eV, in the combined SCAN and SIM mode. The scanning range extended m/z 35-400. The selected ions
200 monitored were m/z 68 (quantifier) and 39 (qualifier) for furan and m/z 72 (quantifier), 44 and 42 (both
201 qualifier) for furan-d_4. MS ion source and quadrupole temperatures were 230 and 150 °C, respectively.
202 Each sample was analyzed in duplicate.
203 The method of internal standard calibration was used to prepare a calibration curve for furan. A serial
204 dilution was made starting from a furan working solution (ca. 0.25 μg/ml in deionized water), resulting
205 in a calibration curve covering the concentration range of 0-50 ng/g purée. The identity of both furan
206 and furan-d_4 was confirmed by calculating the response ratio of the qualifier ions and the quantifier ions
207 according to the guidelines stated in European Commission Decision (European Commission 2002). In

agreement with these guidelines, the method was validated in terms of specificity, recovery, precision (repeatability), decision limit (CCα), and detection capability (CCβ). The decision limit and the detection capability of the procedure were 1.15 ng and 1.86 ng per g purée, respectively. Next to furan, the calibration curve was used to estimate the concentrations of 2- and 3-methylfuran in the samples. According to our own findings (results not shown), the behavior of both methylfurans towards the internal standard furan-d_4 was not significantly different from the behavior of furan. The identity of 2- and 3-methylfuran was confirmed by comparison with retention times of standards and by calculating the response ratio of the qualifier ions and the quantifier ions, as analogous to the identification of furan and furan-d_4. For both methylfurans, the selected ions monitored were m/z 82 (quantifier), 81 and 53 (qualifier).

Results and discussion

Seven vegetable purées (broccoli, orange carrot, pea, potato, pumpkin, red beet and spinach) were selected to investigate the effects of storage and reheating on the furan concentrations of sterilized, vegetable-based foods. Some general trends on the methylfuran concentrations of these vegetable purées are discussed afterwards.

Furan formation during storage of sterilized vegetable purées

Prior to storage, the vegetable purées were subjected to a thermal treatment, aiming at an industrially relevant process value for sterilization (F_0 = 5 min). The sterilized vegetable purées were analyzed for furan and the resulting concentrations are depicted in **Fig. 1**. The range of furan concentrations found after thermal treatment was very comparable to the amounts observed in our previous study (Palmers et al. 2014). Red beet was again the vegetable type which was the most susceptible to furan formation (11 ng/g purée), followed by potato and carrot (both 7 ng/g purée). Broccoli, pea, pumpkin and tomato had a comparable, lower amount of furan (3-5 ng/g purée). To gain insight into the effects of storage on the furan concentrations of vegetable-based foods, the sterilized vegetable purées were stored at two

234 different temperatures (i.e. 4 and 35 °C). The furan concentrations of the vegetable purées stored at the
235 latter temperature are represented in **Fig. 1**, together with the furan concentrations after sterilization. For
236 most of the vegetable purées, the furan concentrations increased during the storage period of 5 months
237 at 35 °C. The extent of this increase was matrix-dependent. A large increase was observed for potato
238 (+283%) and pumpkin (+396%), in which furan concentrations were four to five times higher as
239 compared with the concentrations observed after sterilization. A smaller increase was observed for
240 broccoli (+114%), carrot (+35%) and tomato (+52%). Pea and red beet were the only vegetable purées
241 in which the furan concentrations did not further increase during storage. For red beet, this was a
242 remarkable observation, given the high furan concentration found immediately after sterilization.
243 With the observed increase in furan concentrations during storage, final furan concentrations up to 19
244 (pumpkin) and 28 ng/g purée (potato) were measured. These values were closer to the concentrations
245 found in real vegetable-based products (mean concentrations of 23-24 ng/g for soups, 48-49 ng/g for
246 vegetable-based baby foods) (European Food Safety Authority (EFSA) 2011) than the furan
247 concentrations found immediately after thermal treatment. Therefore, the furan concentrations found in
248 commercial samples can probably be explained by the summed effect of thermal processing and storage.
249 Of course, the specific storage conditions applied for these products will determine to what extent furan
250 forming reactions can occur during storage. The major driving force of these reactions is the storage
251 temperature, but also water activity, pH, packaging and radiation can influence the degradation of food
252 components during storage (Narayan 1997). To evaluate the potential of reducing furan formation
253 during storage, the sterilized vegetable purées were stored at a refrigerated temperature of 4 °C. The
254 storage time was 5 months, like for the vegetable purées stored at 35 °C. The resulting furan
255 concentrations are represented in **Fig. 2**, together with the concentrations after sterilization. The results
256 indicated that the storage temperature plays an important role in the furan formation of vegetable-based
257 products. At 4 °C, the furan concentrations of all the selected vegetable purées remained approximately
258 constant as compared with the values immediately after sterilization. This included potato and pumpkin
259 purée, vegetable purées that were characterized by a large increase in furan concentrations during

storage at 35 °C. In other words, by selecting appropriate (refrigerated) storage conditions for sterilized, vegetable-based products, the furan formation during storage could be reduced to a large extent.

Unlike the thermal treatment, no literature information can be found on the effects of storage for the furan concentrations of vegetable-based food products. At the temperature-abuse conditions applied in this study, there was a clear increase in the furan concentrations of most vegetable purées. The increase in furan concentrations can be attributed to the continued degradation of precursors and intermediate products of furan formation during storage. For potato and pumpkin, the extent of the increase during storage was remarkably large. Since all vegetable purées were stored under the same conditions of reduced oxygen and/or light exposure, it seems unlikely that these extrinsic parameters were responsible for the differences in matrix susceptibility. It is possible that the sterilization treatment generated an additional amount of reactive furan precursors in these matrices (e.g. by thermal degradation of biomolecules such as starch and proteins), which would result in an increased furan formation during storage. In order to control this furan formation in a matrix-specific way, a deeper understanding of the reaction pathways leading to furan formation is indispensable, and should be subject of future research.

Furan formation during reheating of vegetable purées

Most commercially available foods are accompanied by short guidelines on how to prepare that specific product for consumption. For sterilized, vegetable-based foods, these guidelines often include a reheating step. As mentioned in the introduction, reheating of the product is the final preparation step in which the furan concentration is likely to be affected, before consumption. In order to elucidate the general impact of this reheating step on the furan concentrations of sterilized, vegetable-based food products, the thermally treated and stored vegetable purées of the previous section were briefly reheated to 90 °C (3-4 min, depending on the vegetable type). After the reheating step, the vegetable purées were analyzed for furan. In **Table 1**, the results of these analyses are compared with the concentrations of furan after storage, which were already discussed in Section 3.1. In general, furan concentrations decreased as a result of the reheating step. The largest decrease in furan concentrations was observed for

potato (-44%) and pumpkin purée (-70%) stored at 35 °C, which were also the vegetable purées with the highest furan concentrations after storage. When the vegetable purées were characterized by lower initial amounts of furan, the reheating procedure seemed to have a smaller influence on the furan concentrations. As a result, many vegetable purées showed only a small decrease in terms of absolute values (0-2 ng/g purée). Broccoli and tomato purées were almost unaffected by reheating.

Theoretically, a reheating step can influence the furan concentrations of sterilized, vegetable-based food products in two ways (Hasnip et al. 2006; Roberts et al. 2008; Lachenmeier et al. 2009). By heating up the product, both the mobility and the volatility of furan are increased, which can result in evaporative losses of furan to the surroundings. At the same time, an additional amount of furan can be formed due to thermal degradation reactions inside the product. The overall decrease in furan concentrations indicated that for this particular case, evaporation of furan was the dominating event. This was not really a surprise, because the temperature-time conditions applied during the reheating procedure were only a fraction of the heating conditions applied during sterilization. Unless the product is (accidently) exposed to high temperatures or long heating times, the reheating step is not expected to cause a significant increase of the furan concentration in the product. The observed decrease in furan concentrations after reheating of the sterilized or stored vegetable purées corresponds to results reported in the literature. Zoller et al. (2007), Roberts et al. (2008) and Kim et al. (2009) have all demonstrated furan concentrations to be reduced to roughly half the initial concentration after applying different reheating procedures (open/closed heating, microwave/saucepan heating, stirring) to commercially available, ready-to-eat foods. The maximum heating temperature seems to play an important role for the extent of this decrease (Fromberg et al. 2009). When milder procedures of reheating were applied, furan was found to be more persistent (Hasnip et al. 2006; Lachenmeier et al. 2009). The retention of furan in ready-to-eat foods can also be influenced by the presence of lipophilic compounds (such as oils) in the product (Van Lancker et al. 2009), which is an important difference with the vegetable purées used in the present study (no oil/fat added). Furthermore, it is possible that the structural properties of the

311 vegetable purées affect the extent of the furan evaporation (Van Lancker et al. 2009), but this was not
312 clearly observed in the present study.
313
314 ***Trends on the methylfuran concentrations of vegetable purées***
315 Next to furan, the stored and reheated vegetable purées were also analyzed for 2- and 3-methylfuran.
316 Due to the little amount of literature data on the toxicological relevance and the reaction pathways
317 leading to the formation of both methylfurans, the discussion will be limited to a qualitative description
318 of the changes in methylfuran concentrations as influenced by both effects. For most vegetables, the
319 concentrations of both alkylated furan derivatives were very similar to each other. To facilitate their
320 discussion, the concentrations were summed together as a group of methylfuran compounds.
321 The summed methylfuran concentrations of the vegetable purées after sterilization and storage at 35
322 °C are represented in **Fig. 3**. Immediately after the thermal treatment for sterilization, very low amounts
323 of summed methylfuran (1-3 ng/g purée) were found in most vegetable purées. Tomato and carrot purée
324 were the exceptions, with a concentration of 127 and 12 ng/g purée, respectively. A high susceptibility
325 to methylfuran formation for tomato-based products has been observed before (Becalski et al. 2010).
326 For this reason, tomato-based products might be considered as priority matrices with regard to
327 methylfuran mitigation, if deemed necessary. Following storage at 35 °C for 5 months, the
328 concentrations of summed methylfuran in both tomato (-90%) and carrot purée (-88%) decreased, as
329 opposed to the trends observed for furan. The low initial amounts of methylfuran in the other vegetable
330 purées were almost unaffected by the same storage conditions. Similar results were observed for the
331 vegetable purées stored at 4 °C (results not shown), although the decrease in methylfuran concentrations
332 for tomato (-24%) and carrot purée (-55%) was clearly smaller at this lower storage temperature. The
333 decrease in methylfuran concentrations at both storage temperatures is markedly different from the
334 observed increase for furan. 2- and 3-methylfuran seemed to be prone to degradation reactions during
335 storage, whereas furan was considered to be a rather stable molecule based on the results of the storage
336 experiment described above (cf. Section 3.1). Together with the clearly different matrix dependency of

methylfuran formation during thermal treatment for sterilization, these results seem to indicate different reaction pathways for both types of compounds, which would further complicate the mitigation of both compounds in thermally treated foods.

The majority of the vegetable purées tested in the present study showed a (small) decrease in summed methylfuran concentrations after the reheating step prior to consumption (results not shown). Like for furan, the decrease in methylfuran concentrations could most probably be explained by evaporative losses during the reheating procedure and/or partial degradation of both compounds as observed during storage. The decrease in methylfuran concentrations due to the reheating procedure was less pronounced than for furan. Most vegetable purées already had low initial amounts of methylfuran before reheating, and moreover, 2- and 3-methylfuran molecules are less volatile (boiling point around 63-66 °C) as compared with furan (31 °C). With an optimized procedure of reheating, it should be possible to simultaneously reduce furan and methylfuran concentrations of thermally treated foods.

Conclusions

The present study has demonstrated possible furan formation during storage of vegetable-based foods. Following storage for 5 months at 35 °C (temperature-abuse conditions), the furan concentrations of a range of sterilized vegetable purées were increased in a matrix-specific manner. The storage temperature had an important impact on the extent of furan formation during storage. At a refrigerated storage temperature of 4 °C, the furan concentrations of the vegetable purées remained approximately constant for the same period of 5 months. Even though storage at refrigerated temperatures will contribute to the general quality stability of the product, this mitigation strategy might not be a realistic option from an economic point of view. Therefore, research should continue on the elucidation of the relationship between storage conditions and matrix composition on the one side and furan formation on the other side (e.g. by performing kinetic or mechanistic studies). In particular, future experiments should target possible furan formation at room temperatures, which are the typical storage temperatures of sterilized, vegetable-based foods. With regard to the effect of the matrix composition, there are a multitude of

363 factors that can possibly explain the observed matrix-specificity during storage (e.g. precursors, redox
364 condition, pH). A better understanding of the reaction pathways leading to furan, is a logical next step
365 towards control of furan formation in critical matrices, such as the potato and pumpkin purées in the
366 present study. In the search for effective furan mitigation measures that do not compromise general food
367 safety nor quality, the effect of the reheating step prior to consumption was also investigated. As
368 contrary to storage, furan concentrations decreased when the sterilized and stored vegetable purées were
369 subjected to a short reheating step. At the temperature-time conditions applied in this study (3-4 min of
370 reheating to 90 °C), furan was most likely lost due to evaporative losses. Many additional parameters
371 can influence the extent of this evaporation from the product. If further optimized and properly
372 communicated to the general audience, the reheating procedure could result in an easy and reliable way
373 for removing part of the furan concentration in critical products, such as jarred baby foods. In view of
374 their possible toxicological relevance to the risk characterization of furan, the vegetable purées were
375 also analyzed for 2- and 3-methylfuran. Both methylfurans were mainly formed during the thermal
376 treatment for sterilization. Tomato purée was found to be very susceptible to the formation of
377 methylfuran, as opposed to most of the other vegetable purées. An overall decrease in the methylfuran
378 concentrations was observed during storage, which was contrary to the results observed for furan. The
379 concentrations further decreased after reheating of the purées, which could be explained by evaporative
380 losses and/or partial degradation of 2- and 3-methylfuran.
381

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386

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List of tables

Table 1. Change in furan concentrations after reheating of the vegetable purées. For each vegetable purée, a part of the purée after sterilization and the purées stored at 4 and 35 °C was reheated.

Matrix	Treatment	Initial furan conc. (ng/g purée)	Furan conc. after reheating (ng/g purée)	Change (±ng/g purée)	Change (%)
Broccoli	Sterilized (not stored)	2.76	3.38	+0.62	+22
	Stored at 4 °C (5 mos.)	4.18	4.08	-0.10	-2
	Stored at 35 °C (5 mos.)	5.93	5.14	-0.79	-13
Carrot	Sterilized (not stored)	6.81	3.68	-3.14	-46
	Stored at 4 °C (5 mos.)	4.88	4.38	-0.50	-10
	Stored at 35 °C (5 mos.)	9.17	7.39	-1.77	-19
Pea	Sterilized (not stored)	3.38	1.01	-2.37	-70
	Stored at 4 °C (5 mos.)	3.57	3.07	-0.50	-14
	Stored at 35 °C (5 mos.)	3.57	1.11	-2.47	-69

Potato	Sterilized (not stored)	7.36	5.47	-1.90	-26
	Stored at 4 °C (5 mos.)	7.13	3.65	-3.49	-49
	Stored at 35 °C (5 mos.)	28.22	15.75	-12.47	-44
Pumpkin	Sterilized (not stored)	3.88	1.21	-2.67	-69
	Stored at 4 °C (5 mos.)	4.76	3.98	-0.78	-16
	Stored at 35 °C (5 mos.)	19.21	5.61	-13.60	-70
Red beet	Sterilized (not stored)	11.29	9.87	-1.42	-13
	Stored at 4 °C (5 mos.)	11.89	8.63	-3.26	-27
	Stored at 35 °C (5 mos.)	10.93	8.23	-2.70	-25
Tomato	Sterilized (not stored)	4.58	5.60	+1.02	+22
	Stored at 4 °C (5 mos.)	6.39	5.96	-0.43	-7
	Stored at 35 °C (5 mos.)	6.96	6.35	-0.61	-9

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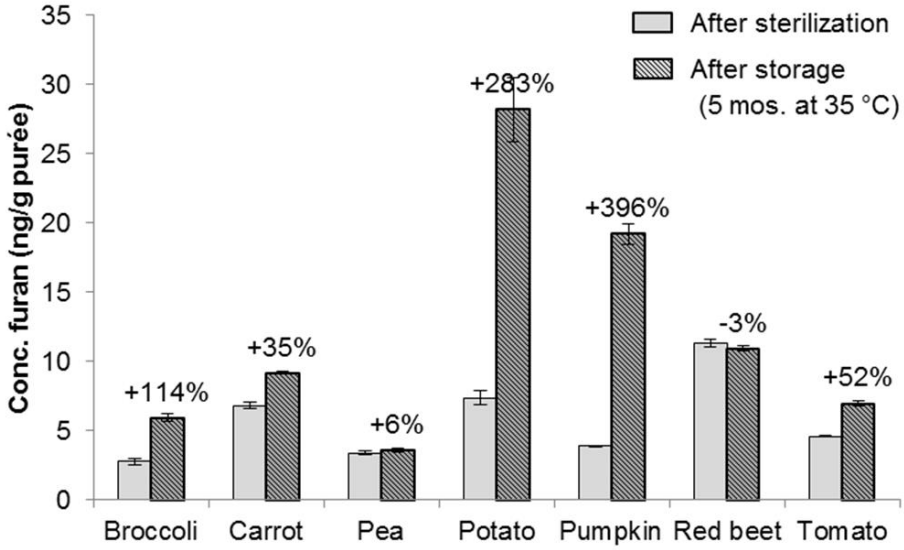

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480 **Fig. 1.** Furan concentrations of the vegetable purées after sterilization (F0 = 5 min) and after 5 months
481 of storage at 35 °C. Data labels indicate the change in furan concentration (in percentages) between both
482 times of measurement.

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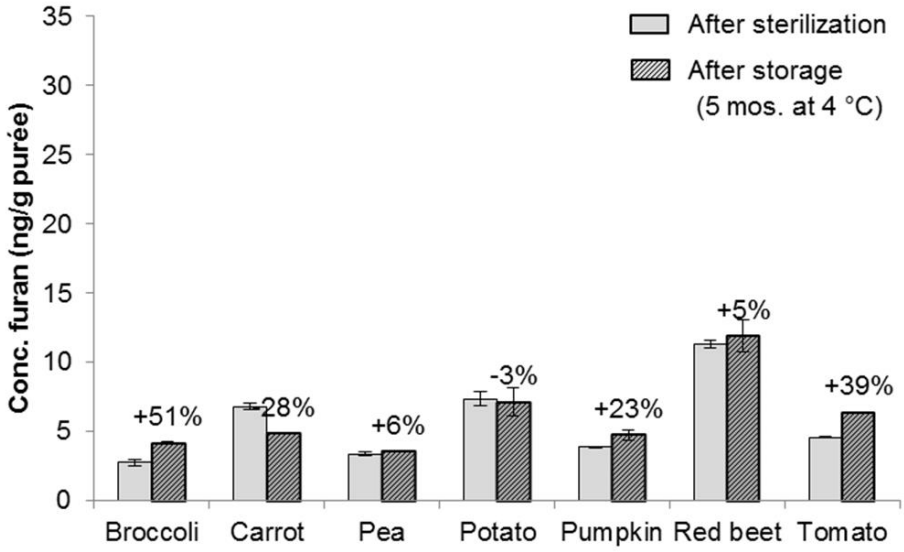

Fig. 2. Furan concentrations of the vegetable purées after sterilization (F_0 = 5 min) and after 5 months of storage at 4 °C. Data labels indicate the change in furan concentration (in percentages) between both times of measurement.

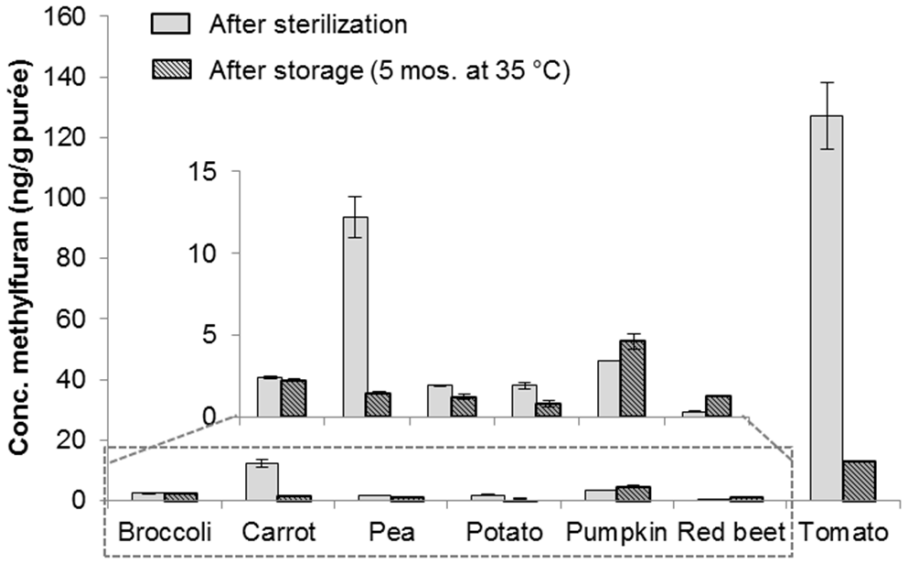

Fig. 3. Summed methylfuran concentrations of the vegetable purées after sterilization (F_0 = 5 min) and after 5 months of storage at 35 °C.